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MINIREVIEW

Mechanisms of Human Papillomavirus-Induced Oncogenesis

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Papillomaviruses are small nonenveloped viruses with 55-nm-diameter icosahedral capsids that contain double-stranded DNA genomes of approximately 8,000 bp. They are widely distributed throughout the animal kingdom, specifically infect squamous epithelia, and cause the generation of warts. An infectious etiology of warts was long suspected and eventually proven in the 19th century. One of the first recorded experimental wart transmission cases in humans appears to have been accidental and was reported in 1845 by a certain Chandler, who "when removing a large acicular condyloma with his instrument injured his assistance beneath the thumbnail. On the injured place there appeared after a short time a wart, which was repeatedly destroyed, but reappeared, until the nail of the injured thumb was removed" (cited in reference 134). Ullmann also noted a similar accidental transmission of laryngeal papillomas and performed self-inoculation experiments with laryngeal papilloma extracts applied to scarified sites on his forearm, and these experiments yielded warts after a lengthy latency period of 9 months (134). Similar inoculation experiments had also been performed with extracts derived from common hand warts (23), and serial inoculation experiments with human subjects were performed (78).

Genital warts and cervical cancer were long regarded as manifestations of then-common venereal diseases such as syphilis and gonorrhea (75). This theory was contested in a rather ghastly paper published in 1917. Extracts of a penile condyloma that was harvested from a young medical student who did not exhibit other overt symptoms of venereal diseases were used to inoculate sites on the forearms of the author and his assistant as well as the genital mucosa of a "virgo intacta." After a period of 2.5 months, the unfortunate female subject developed genital condyloma, and flat warts appeared on the forearms of two male probands (139). These and other experiments led to the realization that genital warts represent distinct disease entities that are caused by a transmissible agent.

The concept that some warts have an inherent propensity for malignant progression was established from studies by Shope, Rous, and others who studied experimental transmission of warts that occur naturally in cottontail rabbits. These investigators discovered that lesions that formed in domestic rabbits after inoculation with cottontail rabbit wart extracts were particularly susceptible to malignant progression (116). Careful transmission studies demonstrated that such extracts caused the emergence of warts only in rabbits and not in other animals, thus illustrating the exquisite species specificity of papillomaviruses (117).

Harald zur Hausen's laboratory was the first to demonstrate that genital warts contain human papillomavirus (HPV) genomes (28, 53). Subsequent low-stringency hybridization experiments with HPV sequences isolated from genital warts performed in his laboratory led to the discovery of related HPV sequences in cervical cancer tissues (38).

HPV AND HUMAN DISEASE

Approximately 200 different HPVs have now been characterized, and new types are regularly added to this list. These viruses can be classified into mucosal and cutaneous HPVs. Within each of these HPV groups, individual viruses are designated high risk or low risk according to the propensity for malignant progression of the lesions that they cause. Most HPVs are low risk and produce localized benign warts that do not undergo malignant progression even if left untreated. Among the cutaneous HPV types, HPV-5 and HPV-8 may be classified as high risk, as they are associated with the development of epidermodysplasia verruciformis (EV), an exceedingly rare skin condition that provided one of the earliest indications that HPVs may contribute to human tumorigenesis (67, 104, 110). EV patients present with flat wart-like cutaneous lesions in early childhood and frequently develop skin cancers later in life, particularly in sun-exposed epithelial sites. There is a clear genetic component to this disease, and the increased incidence of EV-associated cancers in immune-suppressed patients suggests that malignant progression is related to a defect in immune surveillance (3, 112). HPV-5- and HPV-8-related HPVs have been detected in a large percentage of nonmelanoma skin cancers, particularly those that develop in immune-suppressed patients. It has been suggested that these viruses may also contribute to psoriasis and skin tumors in immune-competent individuals. There have been few molecular studies with EV-type HPVs that yield insights regarding the molecular pathways by which these viruses may contribute to skin carcinogenesis (reviewed in references 89 and 109).

Low-risk mucosal HPVs such as HPV-6 and HPV-11 cause genital warts (condyloma accuminata), whereas the high-risk HPVs cause squamous intraepithelial lesions that can progress to invasive squamous cell carcinoma. The vast majority of

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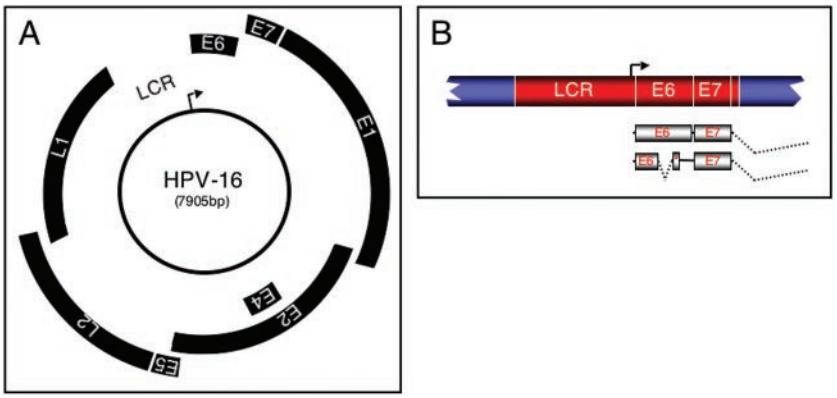

FIG. 1. (A) Schematic representation of the HPV-16 double-stranded circular DNA genome. The early (E) and late (L) genes, as well as the LCR, are shown. The major early promoter (P_{97}) is indicated by an arrow. Transcription occurs from one strand only and is in clockwise orientation in this representation. See the text for details. (B) Schematic structure of the minimal HPV-16 genome fragment (red) retained after integration into a host chromosome (blue). The HPV E6/E7 genes are consistently expressed, whereas the remaining HPV genes are often deleted or not transcribed after integration. Two major HPV RNA species are produced. One transcript has the potential to encode full-length E6 and E7 proteins, and another set of transcripts encodes spliced E6 proteins (designated E6*) and the full-length E7 protein. Most HPV transcripts in cervical cancer cells are spliced downstream of the E7 gene and use cellular splicing and polyadenylation signals. This may cause increased stability of HPV transcripts. See the text for details.

human cervical cancers are associated with high-risk HPV infections. HPV-16 is by far the most prevalent mucosal high-risk HPV type, followed by HPV-18, HPV-31, and others (reviewed in reference 150). Approximately 20% of oral cancers, particularly oropharyngeal carcinomas in patients that lack the classical risk factors of tobacco and alcohol abuse, are also high-risk HPV positive (52). Other anogenital tract malignancies that are also frequently associated with high-risk HPV infections include penile and vulvovaginal cancers (reviewed in reference 26) as well as anal carcinomas, which frequently occur in individuals with human immunodeficiency virus-associated AIDS (reviewed in 82).

Within the family of mucosal HPVs, the low-risk/high-risk classification parallels the transforming potential of the respective viral genomes in cell culture and transgenic mouse models. Hence, much of the molecular research has focused on the analysis of the transforming activities of mucosal high-risk HPVs that are associated with cervical cancer. This review summarizes these insights.

GENOMIC ORGANIZATION AND LIFE CYCLE

Only one of the two strands of the circular papillomavirus DNA genome is actively transcribed. The genome can be divided into three major portions: a ~4-kb early (E) region that encodes nonstructural proteins, a ~3-kb late (L) region that encodes the two capsid proteins, and a ~1-kb noncoding long control region (LCR) that contains a variety of *cis* elements, which regulate viral replication and gene expression. E and L genes are numbered according to size; the higher the number, the smaller the corresponding open reading frame (Fig. 1A).

The papillomavirus life cycle is tightly linked to the differentiation program of the infected epithelium. Papillomaviruses initially infect basal epithelial cells, which constitute the only cell layer in an epithelium that is actively dividing. The nature of the HPV receptor(s) remains unclear, although integrin α4β6 has been implicated (45). Similarly, the processes that mediate virus uptake, decapsidation, and nuclear import of the viral genome remain largely unknown. The viral DNA is maintained at a low copy number in the nuclei of infected host cells as they undergo differentiation and move toward the surface of the epithelium. In terminally differentiated cells, the virus replicates to a high copy number, late genes are expressed, and progeny virus is produced (reviewed in reference 123). HPVs are nonlytic viruses, and progeny virus is shed into the environment as a cargo within epithelial squamae. The HPV E4 protein associates with keratin intermediate filaments, which affects the mechanical stability of the keratin network and may facilitate the release of viral particles (30).

The papillomavirus E1 and E2 proteins each play important roles in viral genome replication. E2 is a DNA binding transcription factor that interacts with $ACCN_6GGT$ motifs in the viral LCR (reviewed in reference 90). High-risk HPV E2 proteins have the capacity to act as transcriptional activators (111), but they function as transcriptional repressors of viral gene expression in keratinocytes (11, 27). In addition to modulating viral gene expression, HPV E2 proteins associate with the viral DNA helicase E1. This interaction is necessary for efficient origin recognition and viral genome replication (21, 22). Papillomavirus E2 proteins also play important roles in viral genome segregation during cell division by tethering viral genomes to mitotic chromosomes (121). The association of E2 with mitotic chromosomes is mediated by interaction with the human bromodomain protein Brd4 (144).

Since HPVs do not encode other enzymes that are rate limiting for DNA replication, production of viral genomes is critically dependent on the host cellular DNA synthesis machinery. Papillomaviruses are replicated in differentiated squamous epithelial cells that are growth arrested and thus intrinsically incompetent to support genome synthesis. Hence, HPVs encode functions that create and/or maintain a replication-competent cellular milieu in infected differentiated keratinocytes. An additional important aspect of the papillomavirus life cycle is the establishment of long-term viral persistence in squamous epithelia, where cells constantly undergo differentiation and differentiated cells are shed. The specific strategies

that high-risk HPVs have evolved to thwart these challenges directly contribute to their oncogenic potential.

HPV GENE EXPRESSION IN CERVICAL CANCERS

One of the key events of HPV-induced carcinogenesis is the integration of the HPV genome into a host chromosome. HPV genome integration often occurs near common fragile sites of the human genome (131), but there are no apparent hot spots for integration and no evidence for insertional mutagenesis (146). Integration follows a more specific pattern with respect to the HPV genome. Expression of the viral E6 and E7 genes is consistently maintained, whereas other portions of the viral DNA are deleted or their expression is disturbed (6) (Fig. 1B). Loss of expression of the HPV E2 transcriptional repressor is significant, as it may result in deregulated HPV E6 and E7 expression. There is also evidence for increased HPV-16 E6/E7 mRNA stability after integration (71), and specific alterations of host cellular gene expression have been detected upon HPV genome integration (1). Cells that express E6/E7 from integrated HPV sequences have a selective growth advantage over cells with episomal HPV genomes (70). The concept that loss of E2 repressor function may be critical for malignant progression is supported by experiments showing that reexpression of E2 in cervical cancer cell lines causes growth suppression (126). These experiments clearly demonstrate that continued E6/E7 expression in cervical cancers is necessary for the maintenance of the transformed phenotype (55, 140).

Integration of the viral genome into a host cell chromosome also leads to loss of E5 expression. In papillomaviruses that cause fibropapillomas, such as bovine papillomavirus type 1, the E5 open reading frame encodes the major transforming viral protein. E5 associates with intracellular membranes and transforms cells by activating receptor tyrosine kinases such as platelet-derived growth factor receptor β through a ligand-independent mechanism (reviewed in reference 29). HPV E5 proteins may have similar activities (87), and disruption of E5 expression affects the life cycle of high-risk HPVs (46, 50). The fact that E5 expression is not generally detected in cervical cancers after viral genome integration demonstrates that E5 is not necessary for the maintenance of the transformed phenotype.

BIOLOGICAL ACTIVITIES OF HPV ONCOPROTEINS

The oncogenic activities of high-risk HPV E6 and E7 genes in tissue culture and transgenic mouse model systems have been documented extensively. Expression of high-risk HPV E6 and E7 genes in primary human keratinocytes effectively facilitates their immortalization (59, 96). When grown under conditions that allow stratification and the formation of skin like structures, high-risk HPV E6/E7 immortalized cells display histomorphological hallmarks of high-grade squamous intraepithelial lesions, well-established precursors of cervical cancers (91). At low passage numbers, however, high-risk HPV immortalized cells are nontumorigenic. They can undergo malignant progression after extended growth in tissue culture or when additional oncogenes such as *ras* or *fos* are expressed (37, 107). The development of cervical cancers in a transgenic mouse model in which HPV-16 E6/E7 is expressed in basal epithelial cells is dependent on long-term exposure to low doses of estrogen (4).

Similarly, progression of high-risk HPV-positive cervical lesions is often a slow process that occurs at a low frequency and requires the acquisition of host cellular mutations (reviewed in reference 150). The rate of spontaneous mutagenesis in normal human cells is exceedingly low, but the expression of high-risk HPV E6/E7 proteins dramatically augments genomic instability (142). Therefore, expression of the high-risk HPV E6/E7 genes not only is necessary for the induction of premalignant alterations but also directly contributes to malignant progression by subverting genomic stability (reviewed in reference 35).

CELLULAR TARGETS OF THE HIGH-RISK HPV E6 AND E7 ONCOPROTEINS

A small set of cellular signal transduction pathways are consistently rendered dysfunctional in the majority of human solid tumors (reviewed in reference 58). Efforts to enumerate the molecular abnormalities in human tumors have more recently been complemented by studies designed to define the minimally necessary series of oncogenic steps necessary to generate fully transformed human epithelial cell lines in vitro. Such experiments have revealed that expression of simian virus 40 (SV40) large tumor antigen (T), SV40 small tumor antigen (t), the catalytic subunit of human telomerase (hTERT), and the H-*ras* oncogene is minimally required to fully transform primary human epithelial cells (reviewed in reference 57). SV40 T functionally inactivates the p53 and retinoblastoma (pRB) tumor suppressors, whereas SV40 t interacts with and inhibits protein phosphatase 2A. The HPV E6 and E7 oncoproteins share functional similarities with SV40 T and inactivate the p53 and pRB tumor suppressors, respectively. In addition, HPV E6 can activate hTERT transcription. Hence, the expression of high-risk HPV E6/E7 oncogenes provides a subset of the minimally required carcinogenic hits for full transformation of primary human epithelial cells.

INDUCTION OF ABERRANT PROLIFERATION BY HIGH-RISK HPV-16 E7 ONCOPROTEINS

HPV E7 proteins are low-molecular-weight proteins of approximately 100 amino acids that have no known intrinsic enzymatic activities. Like other oncoproteins encoded by small DNA tumor viruses, they associate with and modify the functions of cellular protein complexes. The amino-terminal domain of HPV E7 has sequence similarity to a small portion of conserved region 1 (CR1) and to CR2 of adenovirus E1A (Fig. 2A). These sequences are also conserved with SV40 T. The HPV E7 carboxyl terminus contains two copies of a CXXC motif that are separated by a 29-amino-acid spacer. This domain has been implicated in metal binding (8) and may function as a dimerization domain (24, 93). Like adenovirus (Ad) E1A and SV40 T antigen, the HPV E7 proteins interact with the retinoblastoma tumor suppressor protein pRB and the related "pocket proteins" p107 and p130 through a conserved LXCXE sequence within CR2 sequences (39, 40) (Fig. 2A). The pocket proteins regulate the activities of the E2F family of transcription factors that control multiple cell cycle transitions as well as other cellular activities (reviewed in reference 17).

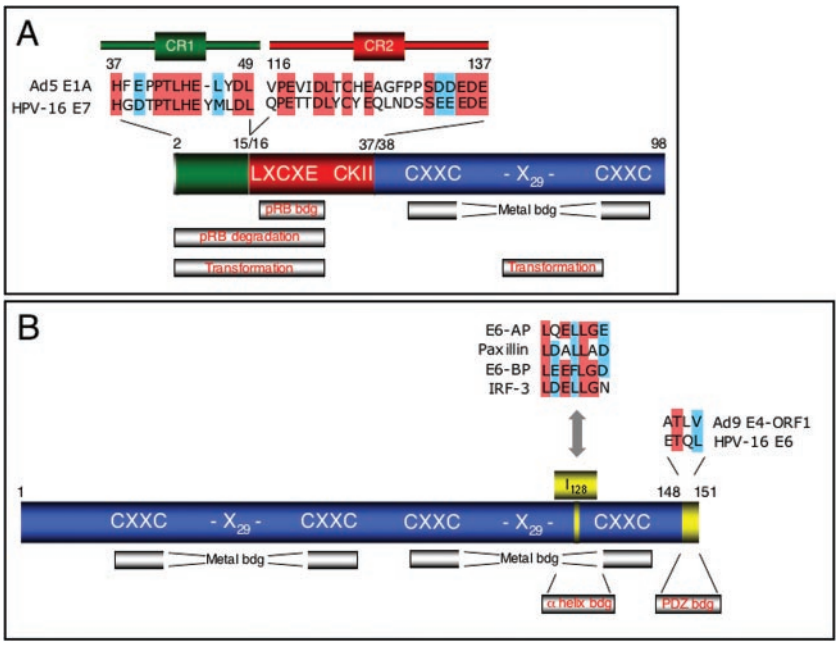

FIG. 2. (A) Schematic representation of the HPV-16 E7 oncoprotein. The amino-terminal 37 amino acid residues have sequence similarity to a portion of CR1 (green) and to CR2 (red) of Ad E1A. Identical and chemically similar amino acid residues between HPV-16 E7 and Ad5 E1A are highlighted by red and blue boxes, respectively. CR1 sequences are necessary for cellular transformation and pRB degradation but do not directly contribute to pRB binding. Sequences in CR2 include the core pRB binding site (LXCXE), which is necessary for cellular transformation, as well as a casein kinase II consensus phosphorylation site (CKII). The E7 carboxyl terminus (blue) contains a metal binding motif and mediates association with multiple host cellular proteins, including histone-modifying enzymes, which may also contribute to cellular transformation. See the text for details and references. (B) Schematic representation of the HPV-16 E6 oncoprotein. The sequence contains two metal binding motifs that are related to the E7 carboxyl terminus (blue). The E6 carboxyl terminus contains a PDZ protein-binding motif (yellow) that is similar to the carboxyl-terminal PDZ binding motif of Ad9 E4 ORF1. Many HPV-16 E6 binding proteins, including E6-AP, paxillin, E6-BP, and IRF-3, contain a conserved α-helical domain and presumably interact with similar E6 sequences. The isoleucine residue at position 128 importantly contributes to interaction with α-helix domains containing E6 binding proteins. Identical and chemically similar amino acid residues are highlighted by red and blue boxes, respectively. See the text for details and references.

The ability of HPV E7, Ad E1A, and SV40 T antigen to associate with pRB is critical for their capacity to generate and/or maintain a host cellular milieu that is conducive to viral genome replication. Consistent with this model, mutation of the LXCXE domain in E7 impedes the HPV life cycle (47, 127). High-risk HPV-derived E7 proteins interact with pRB more efficiently than E7 proteins encoded by low-risk mucosal HPVs (49, 97), and mutations in the LXCXE domain that affect pocket protein association are transformation defective in different assay systems (reviewed in reference 95). High-risk HPV E7 proteins have the unique ability to destabilize the pocket proteins through a proteasome-dependent mechanism (10, 14, 73). In addition to the LXCXE domain, sequences within the amino-terminal CR1 homology domain of high-risk HPV E7 are necessary for the ability to destabilize pocket proteins. High-risk HPV E7 proteins with mutations in the CR1 homology domain are also transformation deficient. Hence, the ability of high-risk E7 proteins to destabilize pocket proteins is critical for cellular transformation (54, 61, 73) (Fig. 2A). In addition to pRB binding and degradation, E7 has other cellular targets that are relevant to cellular transformation. HPV E7 can override the growth-inhibitory activities of cyclin-dependent kinase inhibitors, including $p21^{CIP1}$ (48, 72) and $p27^{KIP1}$ (145). Since these proteins are critical regulators of cell cycle arrest during keratinocyte differentiation (94), their inhibition by E7 may also contribute to the maintenance of a replication-competent cellular milieu in differentiated host epithelial cells (20). A carboxyl-terminal E7 domain that does not contribute to pRB binding and/or degradation is necessary for the ability of E7 to override $p21^{CIP1}$-mediated growth arrest (60). Additional E7-interacting proteins, including transcription factors, cell cycle regulators, and metabolic enzymes, have been isolated by various methods, and many of these candidates appear to associate with carboxyl-terminal E7 sequences (reviewed in reference 95). The biological relevance of many of these interactions, however, remains to be determined. The carboxyl-terminal HPV E7 domain contributes to association with chromatin-modifying enzymes, particularly histone deacetylases and histone acetyl transferases (15). E7 has also been reported to interact with the transcriptional coactivators p300, CBP, and pCAF (5, 12, 64). Similar to the case for the amino-terminal pRB binding site, the integrity of the carboxyl-terminal E7 sequences that have been implicated in histone deacetylase binding are necessary for the viral life cycle (88). Hence, these interactions may contribute to transforming activities of high-risk HPV E7 proteins (Fig. 2A).

ELIMINATION OF TROPHIC SENTINEL SIGNALING BY HIGH-RISK HPV E6 ONCOPROTEINS

The HPV E6 proteins are small proteins of approximately 150 amino acids and contain two domains consisting of paired

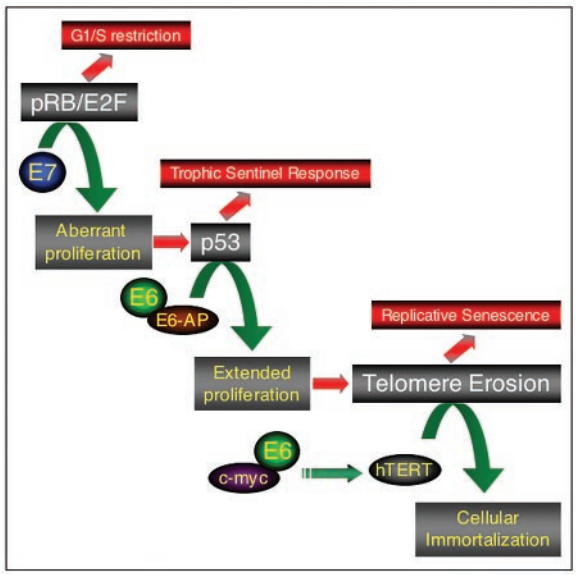

FIG. 3. Schematic outline of critical steps of high-risk HPV-induced carcinogenesis. Inactivation of the pRB and p53 tumor suppressor pathways and expression of the catalytic telomerase subunit hTERT constitute a subset of the steps that have been shown to be necessary for the generation of fully transformed human epithelial cells in vitro. See the text for details.

CXXC motifs that are each related to the E7 carboxyl terminus (25). Induction of aberrant cellular and/or viral DNA synthesis in differentiated keratinocytes that presumably lack environmental mitogen stimulation results in conflicting growth signals. This situation triggers a cellular defense mechanism, the "trophic sentinel response" that eliminates such deviant cells from the proliferative pool through cell type-specific abortive processes, including cell death, differentiation, and senescence (reviewed in reference 43) (Fig. 3). This was originally discovered in transgenic mouse models in which E7 expression caused aberrant proliferation and differentiation, which resulted in cell death (63, 101, 102). Similar to what has been reported for adenovirus E1A (113) and c-*myc* (44), HPV-16 E7-expressing cells are predisposed to cell death when their culture medium is deprived of growth factors (41, 74). This process is p53 dependent, even though many p53-responsive apoptosis regulators are not induced, and cell death appears to be at least in part caspase independent (41, 76).

High-risk HPV E6 proteins eliminate the trophic sentinel response triggered by E7 expression (74) through inactivation of p53. This process is essential for the life cycle of high-risk HPVs (103). High-risk HPV proteins E6 do not directly associate with p53 but form a complex with the cellular E6-AP protein, which is essential for p53 interaction (66). E6-AP is the founding member of the homology to E6 C terminus (HECT) family of E3 ubiquitin ligases (65). E6-AP does not interact with p53 in the absence of E6, and its normal substrates are unknown (9, 124). High-risk E6 proteins retarget E6-AP to induce ubiquitination and rapid proteasomal degradation of p53 (119). HPV-16 E6 proteins may also interact with additional cellular factors that are important for the transcriptional activity of p53, including p300 (105, 147) and the transcriptional coactivator ADA3 (84).

High-risk HPV E6 proteins also have p53-independent transforming activities. These HPV E6 proteins contain a carboxyl-terminal PDZ binding domain (80, 86) (Fig. 2B). PDZ domain proteins act as molecular organizing centers for many cellular signal transduction pathways (reviewed in reference 136). The ability of adenovirus type 9 to induce mammary tumors in rats is linked to the E4 ORF1 protein (68, 69) and its capacity to form complexes with PDZ proteins (86). The high-risk HPV E6 proteins have a marked specificity for particular PDZ domains (129), but the biologically relevant PDZ targets for E6 remain to be determined. The ability of high-risk HPV E6 proteins to associate with PDZ host proteins is relevant to cellular transformation. This relevance has been best illustrated in a transgenic mouse model in which the ability of HPV-16 E6 to induce skin hyperplasias (85) is dependent on the integrity of the carboxyl-terminal PDZ binding domain (98).

A considerable number of additional cellular proteins have been reported to associate with E6. These include the EF-hand calcium-binding protein E6-BP (reticulocalbin 2) (19), the interferon regulatory factor IRF-3 (115), and the focal adhesion protein paxillin (132, 135). Hyperactivity of focal adhesion kinase (FAK) has been detected in cervical cancer and HPV immortalized epithelial cell lines, but the mechanism is unclear (92). Because these and other potential E6 cellular target proteins share a conserved α-helical interaction site for E6 association (18, 42, 133, 135) (Fig. 2B), it has been difficult to determine the relevance of these individual interactions to the biological activities of high-risk HPV E6 proteins.

INDUCTION OF TELOMERASE ACTIVITY BY HIGH-RISK HPV E6 PROTEINS

Each round of DNA replication leads to erosion of the chromosomal telomeric termini. Telomere shortening represents a cell-autonomous mechanism that restricts the proliferative capacity of normal somatic cells. Certain cell types that must undergo a large number of cell divisions, such as stem cells, express telomerase, a ribonucleoprotein that prevents telomere erosion. Ectopic expression of the catalytic telomerase subunit, hTERT, in primary human cells causes life span extension and facilitates immortalization. The majority of human tumor cells are telomerase positive, suggesting that aberrant telomerase activity may be critical for human tumorigenesis. Ectopic hTERT expression also represents one of the obligatory components for the generation of human tumor-like cells in vitro (reviewed in reference 13). In combination with E7, high-risk HPV E6 proteins contribute to immortalization of primary human epithelial cells through the induction of telomerase activity (79, 83). High-risk E6 proteins induce hTERT expression at a transcriptional level (137). The minimal E6 responsive hTERT promoter fragment contains c-*myc*-responsive E-boxes that contribute to E6-mediated transcriptional activation, but E6 does not markedly affect c-*myc* expression or the composition of myc transcription factor complexes (51, 99, 138). There is evidence, however, that E6 di-

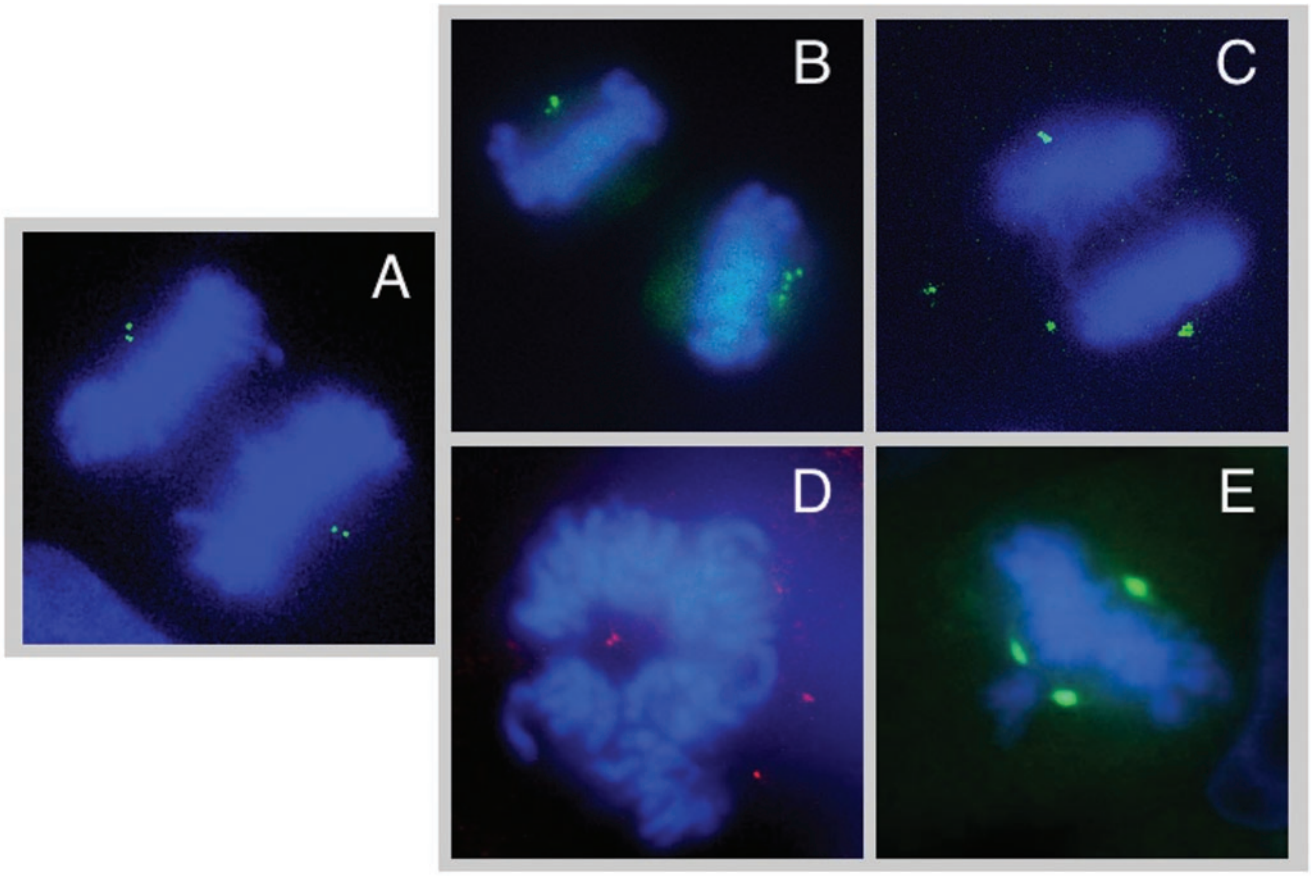

FIG. 4. The HPV-16 E7 oncoprotein contributes to induction of genomic instability by induction of centrosome duplication errors. Shown are examples of different mitotic abnormalities that can be generated by numerical centrosome abnormalities. (A) Normal bipolar metaphase; each mitotic spindle pole body consists of a single centrosome which contains two centrioles. Individual centrioles are visualized by green fluorescent protein (GFP)-centrin fluorescence. (B) Abnormal bipolar mitosis due to centrosome aggregation. Individual centrioles are visualized by GFP-centrin fluorescence. The mitotic spindle pole on the left contains three centrioles, whereas the one on right contains four centrioles that may represent two aggregated centrosomes. There is a chance for nonsymmetrical chromosome segregation upon completion of cell division. (C) Abnormal bipolar mitosis in the presence of multiple individual centrosomes. Individual centrioles are visualized by immunofluorescence by using a centrin-specific antibody. While the majority of the chromosomes are segregated in a bipolar fashion, the centrosomes on the left may interfere with symmetrical chromosome distribution by apparently capturing some chromosomal material. (D) Predominantly monopolar mitosis in the presence of multiple centrosomes. Individual centrosomes are visualized by immunofluorescence by using a γ-tubulin-specific antibody. (E) Tripolar mitotic figures are hallmarks of high-risk HPV-associated cervical lesions. Individual centrosomes are visualized by GFP–γ-tubulin fluorescence.

rectly interacts with c-*myc* and that a c-*myc*/E6 complex activates hTERT expression (138).

HPV ONCOPROTEINS AND GENOMIC INSTABILITY

As outlined in the previous sections, the continued combined expression of high-risk HPV E6 and E7 proteins in cervical cancers causes inactivation of the pRB and p53 tumor suppressor pathways and induces telomerase activity. These signal transduction pathways are disrupted in the majority of human solid tumors (reviewed in reference 58), but they constitute only a subset of the oncogenic hits that are minimally required to generate fully transformed human cells in vitro (reviewed in reference 57). Clearly, additional oncogenic events are necessary in E6/E7 expressing cells to yield full transformation in vivo and in vitro. Consistent with this notion, cervical carcinomas contain chromosomal abnormalities (reviewed in reference 149). Specific gains of chromosome 3q occur at the transition from high-risk-HPV-associated severe dysplasia to invasive carcinoma (56, 62).

Human carcinogenesis has been characterized as a disease of genomic instability (81), and the majority of human solid tumors display evidence of chromosomal aberrations, most notably aneuploidy. Fully transformed human cells generated in vitro retain stability of their genomes (148). Genomic instability therefore does not necessarily arise as a generic manifestation of oncogenic transformation but rather represents a characteristic of tumor cells that enables them to acquire genetic alterations that are necessary for survival and clonal expansion within the rapidly changing microenvironment of an emerging neoplasm (reviewed in reference 16). Hallmarks of genomic instability have even been noted in early premalignant high-risk-HPV-associated lesions. In particular, the presence of tripolar mitotic figures has served as a hallmark to distinguish high-risk-HPV-positive lesions (143).

High-risk HPV E6 and E7 oncoproteins can each indepen-

dently induce genomic instability in normal human cells (142). They cooperate to generate mitotic defects and aneuploidy through the induction of centrosome abnormalities (Fig. 4) in normal human epithelial cells, and the characteristic multipolar mitoses in cervical lesions are caused by centrosome abnormalities (33). In contrast, low-risk HPV E6/E7 proteins are not capable of inducing centrosome abnormalities. Centrosome abnormalities and associated mitotic defects are apparent in cells that, similar to low-grade HPV-associated lesions, express episomal HPV-16 at a low copy number (32), and their incidence increases in cells with integrated HPV (108). Centrosome abnormalities have also been detected in cervical (7, 114) and skin lesions that arise in HPV-16 E6- and/or E7-expressing transgenic mice (118).

In many tumors, centrosome abnormalities emerge as a consequence of cytokinesis and/or cell division defects, thus occurring mostly in cells that have also accumulated nuclear abnormalities (reviewed in reference 122). In stark contrast, however, HPV E7 expression induces primary centrosome and centriole duplication errors in normal diploid cells (31). The detailed molecular mechanisms of this activity of E7 remain to be determined. This activity is at least in part independent of the ability to target pRB family members, since the expression of HPV-16 E7 causes an increased incidence of centrosome abnormalities in mouse embryo fibroblasts that lack pRB, p107, and p130 expression (34). Thus, HPV-16 E7 may act as a mitotic mutator which by increasing the likelihood of mitotic errors during each round of cell division provides the necessary genomic plasticity for the acquisition of additional cellular mutations that contribute to malignant progression (reviewed in reference 35). HPV oncoprotein-expressing cells also exhibit centrosome-independent manifestations of genomic instability. These manifestations include anaphase bridges that may be caused by double-strand DNA breaks as well as lagging chromosomal material (36). HPV-16 expressing cells have a higher propensity for integration of plasmid DNA (77). The observed incidence of double-strand DNA breaks in HPV-16 E6/E7-expressing cells may provide for a mechanistic rationalization of this observation and may facilitate HPV genome integration that often accompanies malignant progression. In addition, high-risk HPV E6 and E7 proteins eliminate multiple mitotic checkpoints and/or the tetraploidy checkpoint that normally blocks tetraploid cells from reentering the cell division cycle (128, 130). Genomic analyses have offered additional evidence for the dysregulation of mitotic pathways in cervical cancer and high-risk-HPV-expressing cell lines (106, 125).

CONCLUDING REMARKS

The transforming activities of high-risk HPVs represent a consequence of a viral replication strategy that is driven by the necessity to replicate viral genomes in suprabasal, normally growth-arrested differentiated epithelial cells and to establish long-term maintenance in a tissue in which individual cells are rapidly turned over and shed. Carcinogenic progression of high-risk-HPV-infected cells is an abortive, terminal event, since most cancer cells contain integrated HPV genomes and do produce viral progeny. If the integration of high-risk HPV genomes indeed represents a consequence of HPV E6/E7-induced genomic instability, it appears that such a replication strategy might put high-risk HPVs at an evolutionary disadvantage compared to the low-risk HPVs that infect the anogenital tract mucosa. Low-risk HPVs effectively induce epithelial hyperplasia and produce copious amounts of progeny virus. Low-risk HPV E6 and E7 proteins critically contribute to viral life cycle (100), but they have a substantially lower transforming activity and do not induce genomic instability. Low-risk HPV E7 proteins bind to pRB at a decreased efficiency (49, 97) and do not induce pRB destabilization (54). Low-risk HPV E6 proteins do not efficiently interact with p53 (141) and are incompetent for p53 degradation (120). They lack carboxyl-terminal PDZ binding domains (80, 86) and do not induce telomerase activity (83). Consequently, it is tempting to speculate that life cycles of mucosal high-risk and low-risk HPVs differ fundamentally. High-risk HPVs can frequently persist in an infected host cell at a low copy number for decades, often without causing clinically overt lesions. This is remarkable given that squamous epithelial cells are turned over very rapidly. A relatively small number of basal epithelial cells have characteristics of stem cells and constantly produce differentiation-competent squamous epithelial cells to maintain the integrity of the epithelium throughout the life of the organism (reviewed in reference 2). One might envision a scenario in which high-risk HPVs have evolved to be able to maintain their infected host cell in a stem cell-like state in order to establish a persistent infection. The high-risk HPV-specific biological activities of E6 and E7 may reflect this strategy. Low-risk HPVs may have evolved a life cycle that is optimized to rapidly produce copious amounts of progeny virus and readily form large productive lesions to maximize transmission of the virus to a new host. Such a model may predict that different HPVs may infect distinct target cells and that there may be differences in the persistence of viral genomes in infected host cells.

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